BLOCKAGE AND RECOVERY OF NITRIFICATION IN SOILS EXPOSED TO ACETYLENE[1]

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ABSTRACT.—Acetylene gas is very useful in laboratory and in situ assay procedures for nitrogen fixation and denitrification. There is concern, however, that measurements of denitrification may be underestimated because nitrification, a major source of nitrate, is inhibited by C_2H_2. The objective of this study was to examine the effects of C_2H_2 partial pressure and length of exposure time on nitrification in soils. Acetylene partial pressures of 0.1, 1.0, and 10.0 kPa were found to effectively inhibit nitrification in soil samples incubated in the laboratory. Both the partial pressure of C_2H_2 and the length of exposure time were found to affect the recovery time of nitrification in soil samples. Nitrification recovered within seven days in samples exposed to 0.1 and 1.0 kPa C_2H_2 for only 24 hours. The recovery of nitrification in samples exposed to 10.0 kPa C_2H_2 for 24 hours or to 0.1 and 1.0 kPa C_2H_2 for 216 hours was delayed for an additional seven days, however.

Acetylene gas has been effectively used in laboratory and in situ techniques for the measurement of both nitrogen fixation and denitrification. The use of acetylene (C_2H_2) is attractive due to the low cost and high sensitivity of the procedures. For N_2 fixation studies, C_2H_2 is used to saturate enzymes responsible for fixation; in the process, C_2H_2 is reduced to ethylene (C_2H_4). Ethylene can be readily detected by gas chromatography and N_2 fixation rates estimated (Hardy et al. 1968, Bergerson 1980). Acetylene has an inhibitory effect on the bacterial enzymes that reduce N_2O to N_2; therefore, C_2H_2 has been used in denitrification studies (Balderson et al. 1976, Yoshinari et al. 1977). In the presence of C_2H_2 partial pressures greater than 0.1 kPa, N_2O is the sole gaseous product of denitrification and is detectable by gas chromatography.

Concerns have been expressed about the use of C_2H_2 in denitrification studies, because C_2H_2 has been found to be an effective nitrification inhibitor at a partial pressure of 0.01 kPa (Walter et al. 1979, Berg et al. 1982). In some situations denitrification measurements may be affected by C_2H_2, because nitrification, a major source of NO_3^-, is inhibited. This is not a problem with short-term laboratory incubations or experiments that involve the addition of nitrate to the soil, but it may be inappropriate to use acetylene in experiments where NH_4^+ is used as the starting point in denitrification studies or in long-term experiments where mineralization and subsequent nitrification could be expected to produce significant nitrate.

Researchers have studied nitrification in the presence of C_2H_2 partial pressures ranging from 1,000 to 0.01 Pa (Walter et al. 1979, Berg et al. 1982). The minimum effective partial pressure that inhibited nitrification was 10 Pa. However, partial inhibition was found at 0.1 Pa (Berg et al. 1982). These researchers reported that the inhibitory effects of the low C_2H_2 levels (1,000 to 10 Pa) ceased within 7 to 10 days of removal of C_2H_2.

Acetylene partial pressures of 10 kPa are routinely used in nitrogen fixation studies (Hardy et al. 1968, Bergerson 1980) and in laboratory denitrification studies (Yoshinari et al. 1977, Terry and Tate 1980a, 1980b, Terry et al. 1981). Field measurement of denitrification by the C_2H_2 inhibition technique involves introduction of C_2H_2 to the soil atmosphere through perforated tubing or C_2H_2 treated irrigation water (Ryden, *I., Laboratory evaluation*, 1979; Ryden, II., *Development and application*, 1979, Rolston et al. 1982, Hallmark and Terry 1985). It is likely, with these procedures, that C_2H_2 partial pressures in excess of 10 kPa will exist in portions of the soil atmosphere that will maintain minimum effective levels of 0.1 kPa throughout the soil atmosphere.

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TABLE 1. The properties of soils used in this investigation.

Soil series	pH	Sand	Silt	Clay	Organic-C	Total-N
				$g\ kg^{-1}$		
Timpanogos	7.9	348	309	343	12.5	1.27
Woodrow	8.1	239	390	375	8.8	1.40

The effects of C_2H_2 partial pressures greater than 1 kPa on nitrification and the resumption of nitrification following exposure have not been studied. The objective of this research was to determine the effects of various C_2H_2 partial pressures and length of C_2H_2 exposure time on nitrification in soils.

MATERIALS AND METHODS

Soils used in this investigation were Timpanogos clay loam (a fine, loamy, mixed mesic Calcic Argixeroll) collected at the Brigham Young University Agriculture Station near Spanish Fork, Utah, and Woodrow clay loam (a fine silty, mixed mesic Xeric Torrifluvent), collected at Camp Floyd State Park, Fairfield, Utah. The properties of the soils used in this study are presented in Table 1. Air-dried soil samples were analyzed for total N by the micro-Kjeldahl method of Bremner (1965) and for organic C by the method of Allison (1965). The soil pH was measured by glass electrode on a 1:1 soil:water ratio.

Duplicate moist soil samples, the equivalent of 10.0 g dry weight, were placed into 120 ml serum bottles and sealed with septum stoppers. The samples were brought to approximately −0.333 MPa matric potential by addition of 1.25 ml of a solution containing 3.96 g $(NH_4)_2\ SO_4\ L^{-1}$ to equal an application rate of 100 mg N kg^{-1} soil. The soils were preincubated for 24 hours before treatment with C_2H_2.

To compare the inhibitory effects of various levels of C_2H_2 with those of the commercial nitrification inhibitor, nitrapyrin, soil samples were continuously exposed to C_2H_2 partial pressures of 0.1, 1.0 or 10.0 kPa. Impurities were removed from C_2H_2 by passage of the gas through concentrated H_2SO_4 and water traps (Hardy et al. 1968). Nitrapyrin was dissolved in the ammonium solution and added to the soil samples at the rate of 2 mg kg^{-1}. The continuously exposed samples were unsealed, aerated for 10 minutes once a week, then reexposed to acetylene, thus eliminating anaerobic conditions. Nitrapyrin samples were also aerated for 10 minutes once a week.

To examine the effects of various partial pressures of acetylene on nitrification and the recovery of nitrification following exposure, acetylene was added at rates of 0, 0.1, 1.0, and 10.0 kPa for a period of 24 hours and then removed by flushing the incubation vessels with air. The samples were then incubated at 21 C and continuously aerated with laboratory air at 100% relative humidity using a manifold delivery system and an aquarium air pump. The air flow rate for each sample was 0.5 mL s^{-1}.

To determine the effects of length of acetylene exposure time on nitrification, soil samples were exposed to 0.1, 1.0, or 10.0 kPa C_2H_2 for 24 or 216 hours. Following exposure, acetylene was removed by flushing with air and the samples were then continuously aerated.

Sufficient samples were prepared to allow for duplicate analyses at 0, 7, 14, 21, and 28 days. At the end of incubation inorganic N was extracted from the samples with 50 mL of 2M KCl and NH_4^+, NO_2^-, and NO_3^- were determined by the steam distillation procedure of Bremner and Keeney (1965).

RESULTS AND DISCUSSION

The effectiveness of C_2H_2 and the commercial nitrification inhibitor, nitrapyrin, on inhibition of nitrification in Timpanogos clay loam was tested. Nitrapyrin, and C_2H_2 at partial pressures ranging from 0.1 to 10.0 kPa, effectively inhibited nitrification in this soil throughout the 28-day incubation (Table 2). The soil samples were preincubated for 1 day to allow the $(NH_4)_2SO_4$ or $(NH_4)_2\ SO_4$ + nitrapyrin solutions to equilibrate with the soil prior to C_2H_2 addition and incubation. The difference in the NH_4^+-N levels between the nitrapyrin and C_2H_2 treatments at day 0 indicated that approximately 20% of the added

TABLE 2. The effects of various acetylene partial pressures and nitrapyrin on nitrification in Timpanogos clay loam.

C_2H_2 Partial pressure #	Form of N	Days of incubation 0	7	14	21	28
kPa		Inorganic N mg kg^{-1}				
0.1	NH_4^+	79	79	82	83	81
	$NO_3^- + NO_2^-$	60	62	60	61	60
1.0	NH_4^+	80	81	83	84	84
	$NO_3^- + NO_2^-$	60	61	62	61	62
10.0	NH_4^+	80	80	81	84	87
	$NO_3^- + NO_2^-$	59	61	61	59	61
NITRAPYRIN	NH_4^+	98	95	97	97	n.d.*
	$NO_3^- + NO_2^-$	55	51	56	53	n.d.*

*n.d. = not determined
#Except Nitrapyrin

TABLE 3. The effects of a 24-hour exposure to C_2H_2 partial pressures ranging from 0 to 10 kPa on nitrification in Timpanogos cl.

C_2H_2 Partial pressure	Form of N	Days of incubation 0	7	14	21	28
kPa		Inorganic N mg kg^{-1}				
0	NH_4^+	52	0	0	0	0
	$NO_3^- + NO_2^-$	83	140	147	148	150
0.1	NH_4^+	78	0	0	0	0
	$NO_3^- + NO_2^-$	61	146	146	152	148
1.0	NH_4^+	70	0	0	0	0
	$NO_3^- + NO_2^-$	63	144	144	152	156
10.0	NH_4^+	68	45	2	0	0
	$NO_3^- + NO_2^-$	63	93	140	150	155

NH_4^+-N was nitrified during preincubation. The finding that C_2H_2 partial pressures ranging from 0.1 to 10.0 kPa inhibited nitrification concurred with earlier work of Walter et al. (1979) and Berg et al. (1982), who showed that C_2H_2 partial pressures of 1.0 and 0.1 kPa effectively inhibited nitrification in soils.

The effects of 24 hours of exposure to C_2H_2 partial pressures ranging from 0 to 10.0 kPa on subsequent changes in NH_4^+-N and $(NO_3^- + NO_2^-)-N$ concentrations in the Timpanogos soil are shown in Table 3. The zero time of incubation followed the 24-hour preincubation and the 24 hours of exposure of C_2H_2. Nitrification continued in the control samples (0 kPa) during the 24-hour period that the remaining treatments were exposed to C_2H_2. For this reason more $(NO_3^- + NO_2^-)-N$ had accumulated in the control samples during preincubation. During the first week if incubation following removal of C_2H_2, nitrification of added NH_4^+-N was complete in all samples except those treated with 10.0 kPa. Nitrification in the samples exposed to 10.0 kPa C_2H_2 for 24 hours was slowed for approximately two weeks due to the lingering effects of acetylene exposure. There were no differences in the NH_4^+-N and $(NO_3^- + NO_2^-)-N$ concentrations during incubation of the 0.1 and 1.0 kPa treatments. During the first week following removal of C_2H_2 from the samples, the nitrification rate in samples exposed to 0.1 and 1.0 kPa C_2H_2 was 12 mg N kg^{-1} day^{-1} compared to 4.3 mg N kg^{-1} day^{-1} in those treated with 10 kPa.

Samples of the Woodrow clay loam were incubated aerobically following a 24-hour exposure to, and subsequent removal of, C_2H_2 partial pressures ranging from 0 to 10.0 kPa. The effects of this brief exposure on subsequent changes in NH_4^+-N and $(NO_3^- + NO_2^-)-N$ concentrations are shown in Table 4. During the first week following C_2H_2 removal, the nitrification rates for 0, 0.1, 1.0, and 10.0 kPa C_2H_2 treatments were 5.2, 5.7, 2.1, and 3.6 mg N kg^{-1} day^{-1}, respectively. Accumulation of $(NO_3^- + NO_2^-)-N$ in the samples exposed to 1.0 and 10.0 kPa was slower during the first week of incubation.

Nitrification proceeded in the Woodrow cl, an unfertilized rangeland soil, at a slower pace

TABLE 4. The effects of a 24-hour exposure of C_2H_2 partial pressures ranging from 0 to 10 kPa on nitrification in Woodrow cl.

C_2H_2 Partial pressure	Form of N	Days of incubation 0	7	14	21	28
kPa		Inorganic N mg kg^{-1}				
0	NH_4^+	79	31	0	0	0
	$NO_3^- + NO_2^-$	19	56	105	106	114
0.1	NH_4^+	87	54	5	0	0
	$NO_3^- + NO_2^-$	12	52	98	104	111
1.0	NH_4^+	83	70	19	0	0
	$NO_3^- + NO_2^-$	12	27	82	101	111
10.0	NH_4^+	83	62	18	2	0
	$NO_3^- + NO_2^-$	13	38	83	93	110

TABLE 5. The effects of C_2H_2 partial pressure and length of exposure time on nitrification in Timpanogos cl.

C_2H_2 Partial pressure	Exposure time	Form of N	Days of incubation 0	7	14	21	28
kPa	Hours		Inorganic N mg kg^{-1}				
10	24	NH_4^+	68	45	2	0	0
		$NO_3^- + NO_2^-$	63	93	140	151	155
	216	NH_4^+	81	48	1	0	0
		$NO_3^- + NO_2^-$	59	89	149	149	148
1.0	24	NH_4^+	70	0	0	0	0
		$NO_3^- + NO_2^-$	63	144	145	153	156
	216	NH_4^+	81	47	1	0	0
		$NO_3^- + NO_2^-$	60	95	139	148	158
0.1	24	NH_4^+	78	0	0	0	0
		$NO_3^- + NO_2^-$	61	146	146	153	148
	216	NH_4^+	80	47	1	0	0
		$NO_3^- + NO_2^-$	61	94	148	152	158

than in the Timpanogos cl, a fertilized cropped soil. The results reported above indicate that brief exposure (24 hours) to C_2H_2 partial pressures ranging from 0.1 to 1.0 kPa have little effect on nitrification in these soils. Brief exposure of soil to 10.0 kPa C_2H_2 slowed subsequent nitrification for as long as two weeks, however.

The effects of length of C_2H_2 exposure time on recovery of nitrification in Timpanogos cl are shown in Table 5. Nitrification rates in soil samples treated with 10.0 kPa acetylene were equivalent during the first week of incubation following C_2H_2 removal whether exposed to the gas for 24 or 216 hours. Nitrification was nearly complete within one week in samples exposed to 0.1 or 1.0 kPa for only 24 hours. Nitrification rates in samples exposed to 0.1 and 1.0 kPa C_2H_2 for 216 hours were much slower, however. The effects of lengthy exposure (216 hours) to C_2H_2 partial pressures of 0.1 and 1.0 kPa on subsequent nitrification were similar to the effects of brief exposure (24 hours) to 10.0 kPa.

Walter et al. (1979) reported that nitrification rates in soil samples exposed to C_2H_2 partial pressures ranging from 0.1 to 1.0 kPa for 24 hours returned to those of control samples after an 8 to 10 day lag period. Similar results were reported by Berg et al. (1982), who exposed soil samples to 0.01 kPa C_2H_2 for seven days. They reported that rates of nitrate production were similar to the rates in control samples seven days after C_2H_2 removal.

The findings of this experiment indicate that both the partial pressure of C_2H_2 and the length of exposure time affect the recovery time of nitrification in soil samples. Exposure of Woodrow cl to 0.1 and 1.0 kPa C_2H_2 for 24 hours slowed nitrification for approximately seven days. Exposure of Timpanogos cl to 0.1 and 1.0 kPa C_2H_2 for 24 hours had little effect on nitrification once the inhibitor was removed. Exposure of this soil to 0.1 and 1.0 kPa acetylene for 216 hours slowed nitrification for approximately seven days, however. These levels of C_2H_2 exposure are commonly used in laboratory and in situ denitrification studies (Yoshinari et al. 1977, Ryden, *II., Development and application*, 1979; Rolson et al. 1982, Ryden and Dawson 1982). The recovery of nitrification in Timpanogos soil exposed to 10.0 kPa C_2H_2 was delayed for at least seven days whether the samples were exposed to the gas for 24 or 216 hours. Acetylene partial pressures of 10.0 kPa have been used in denitrification studies and in studies of concurrent denitrification and nitrogen fixation (Terry and Tate 1980a, 1980b, Yoshinari et al. 1977).

Problems with the use of C_2H_2 in denitrification studies would likely be encountered in soils where the nitrate supply is limited by nitrification. Ryden (1982) reported that denitrification was underestimated in soil samples incubated in the laboratory in the presence of C_2H_2 for 168 hours. Nitrate became exhausted in the samples during incubation. Problems with nitrification inhibition in denitrification studies may be avoided in laboratory studies by adding supplemental nitrate and/or adopting short incubation periods (<24 hours) (Terry and Tate, 1980b). In the design of field studies on denitrification, it would be wise to rotate study sites every 7 to 14 days to allow nitrification to proceed in the soil. The use of sites previously exposed to C_2H_2 should be avoided for 14 to 21 days because of the slow recovery of nitrification following prolonged exposure to C_2H_2.

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